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Disruption of Adenovirus Type 7 by Lithium Iodide Resulting in the Release of Viral Deoxyribonucleic Acid

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Adenovirus type 7 exposed to solutions of LiI was progressively converted into slower sedimenting deoxyribonucleic acid (DNA)-containing particles, and, ultimately, under proper conditions, DNA free or almost free from protein was released from the virus. The degree of viral degradation was dependent on the time of treatment, on the temperature, and on the concentration of the reagent.

Internal nucleoprotein cores are released from adenoviruses treated by acetone (8), heat (19), formamide (20; A R. Neurath et al., *in preparation*), 5 M urea (12), and freezing and thawing (17). None of these treatments results in the release from the virus particles of free viral deoxyribonucleic acid (DNA). The adenovirus capsid is also disrupted into its individual capsomers when exposed to 6 M LiCl (9). Since this reagent also causes cleavage of ribosomal proteins from ribosomal ribonucleic acid (RNA; 15) and was successfully used for the isolation of infectious RNA from cucumber mosaic virus (2) and from potato virus X (1), it seemed to be of interest to study in detail the effect of lithium halides on adenoviruses and to attempt separating viral DNA from capsid and internal core proteins. Lithium iodide was chosen for such studies, since it was expected that it would be more efficient than LiCl (21) and less detrimental to viral proteins than sodium perchlorate, which has been used for the isolation of nucleic acids from some bacteriophages (3, 4). Studies on the effect of LiI on adenovirus type 7, published recently in an abbreviated form (A. R. Neurath, J. T. Stasny, and B. A. Rubin, Bacteriol. Proc., p. 181, 1969), show that this reagent disrupts the viral capsid and the core, releasing viral DNA.

MATERIALS AND METHODS

Virus. Adenovirus type 7 was propagated in human diploid WI-38 cells under conditions described previously (14). Isotopically labeled virus was prepared by infecting human embryonic kidney cells in monolayers (supplied by Microbiological Associates, Inc., Bethesda, Md., in "A" size bottles) with human diploid cell-grown virus at a multiplicity of approximately 200 infectious virus particles per cell. Ninety minutes later, the culture fluids were removed and replaced by 50 ml of diploid cell growth medium (Grand Island Biological Co., Grand Island, N.Y.). Four and one-half hours later, the medium was again exchanged with the same type of medium (50 ml) supplemented with 1 μc of thymidine-*methyl*-^3H per ml (specific activity, 6 c/mmole) or with 1 μc of a ^{14}C-amino acid mixture per ml (algal hydrolysate; specific activity, 1 mc/mg). In the latter case, the concentration of unlabeled amino acids in the medium was reduced 10 times. Both isotopes were obtained from International Chemical & Nuclear Corp., Irvine, Calif. The infected cells were kept at 37 C until a complete cytopathogenic effect appeared. The cells and tissue culture fluids were then frozen and thawed four times. After clarification by low-speed centrifugation, 1 part of tissue culture material was mixed with 0.6 part of a saturated solution (20 C) of ammonium sulfate. The precipitate which formed was pelleted by centrifugation at 3,000 $\times$ *g* for 30 min and then redissolved by 0.01 M tris(hydroxymethyl)aminomethane (Tris)-acetate buffer (*p*H 7.0) in about 0.01 of the original tissue culture fluid volume. Both virus and soluble viral components are concentrated by this procedure. To concentrate further and partly purify the virus, 1-ml samples of the virus suspension were centrifuged for 30 min at 24,000 $\times$ *g* in an SW 65 rotor (Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.). The pellets were resuspended in appropriate buffer solutions and used for experiments that same day. When the ^3H-thymidine-labeled virus preparations were submitted to rate zonal or to isopycnic density gradient centrifugations, a single peak of radioactivity appeared in the gradients in a position expected for intact adenovirus, except that a minor fraction of radioactivity appeared at the top of the gradients which represented products of spontaneous breakdown of the virus.

Biological determinations. Titration of infectious

virus, complement-fixing (CF) antigens, complete hemagglutinins (HA), and incomplete hemagglutinins (IHA) were performed as described previously (14).

Rate zonal centrifugations. Rate zonal centrifugation was performed with 4-ml linear sucrose gradients [10 to 25% sucrose in phosphate-buffered saline (0.075 M phosphate and 0.072 M NaCl, *p*H 7.2)] in centrifuge tubes for the SW 65 rotor. Samples of 0.2 to 0.4 ml were layered on the top of the gradients. The time and speed of centrifugation differed in various experiments and will be given for each case. Disodium ethylenediaminetetraacetate (EDTA) and sodium arsenite (both 0.033 M) were incorporated into the gradients when studies on adenovirus DNA were performed. Approximate values of sedimentation coefficients ($S_{w,20}$) were calculated as mentioned in a preceding paper (14). Consecutively numbered fractions were collected from the bottom of the tubes.

Isopycnic density gradient centrifugation. Samples (1 ml) of isotopically labeled adenovirus treated by LiI were layered over 2.5 ml of a saturated solution of CsCl in 0.01 M Tris-acetate-0.01 M EDTA-0.01 M sodium arsenite (*p*H 7.0) and centrifuged at 109,000 × *g* for 48 to 67 hr in the SW 65 rotor.

Measurement of radioactivity. Samples of 0.02 to 0.5 ml were placed in vials containing 18 ml of a solution with the following composition: 100 g of naphthalene, 10 g of 2,5-diphenyloxazole, 250 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene, 200 ml of methanol, and 1,000 ml of *p*-dioxane (spectro-quality). Radioactivity was measured in a Packard 3375 Tri-Carb liquid scintillation counter.

Treatment of virus and soluble viral components with LiI. To study the inactivation of adenovirus infectivity, the inactivation of HA, and the degradation of virus particles by LiI under different conditions, appropriate volumes of the viral preparation and of the reagent (200 parts of 33% LiI mixed with 1 part of 0.05 M $Na_2S_2O_3$ just before use) were mixed and incubated at a chosen temperature. Samples were withdrawn at suitable time intervals and either diluted in 0.9% NaCl or dialyzed against the same solution. For studies on DNA released from the virus, 0.033 M Tris-acetate-0.033 M EDTA-0.033 M sodium arsenite (*p*H 7.0) was used for both suspension of the virus particles before treatment and for dialysis after treatment.

Treatment of adenovirus DNA with exonuclease I. DNA released from the virus and banded in CsCl gradients (Fig. 4) was dialyzed against 0.066 M glycine-0.0066 M $MgCl_2$ buffer (*p*H 9.2) overnight. Part of the dialyzed sample was immersed in a boiling-water bath for 5 min and rapidly cooled in ice to denature the DNA. Samples (0.5 ml) of the original and heat-denatured DNA were treated with 10 or 80 units of exonuclease I (Worthington Biochemical Corp., Freehold, N.J.) for 30 min at 36 C. Then, 100 μg of bovine serum albumin (in 0.1 ml of the above glycine buffer) and 0.15 ml of 50% trichloroacetic acid were added to the DNA samples (0.5 ml) that had either been treated with the enzyme or had not been treated. The precipitates which formed were collected on 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). Both the filtrates and the filters (which readily dissolved in the scintillation fluid) were counted for radioactivity. The fraction of digested DNA in each sample was expressed as the ratio of counts in the filtrate divided by the total counts in the filtrate and precipitate collected on the filters.

Adsorption of labeled virus on monkey red blood cells (RBC). Samples (1 ml) of ^{3}H-thymidine-labeled adenovirus, either untreated or treated with LiI for 30 min at 36 C and subsequently dialyzed against 0.9% NaCl, were mixed with 0.1 ml of packed rhesus monkey RBC and incubated for 30 min at 36 C. The cells were then sedimented by centrifugation and solubilized in a 1% solution of Triton X-100 (alkyl phenoxy polyethoxy ethanol; Rohm & Haas, Philadelphia, Pa.). The solubilized cells and the supernatant solutions were counted for radioactivity. The counts were corrected for quenching, and the percentage of label (i.e., virus) adsorbed on RBC was calculated.

Electron microscopy. DNA recovered from CsCl gradients after isopycnic centrifugation of virus treated with 22% LiI for 30 min at 36 C was dialyzed against 0.033 M Tris-acetate-0.033 M EDTA-0.033 M sodium arsenite buffer and then spread on a monolayer of cytochrome *c* and shadow casted as described by MacHattie et al. (11). An RCA EMU-3H electron microscope employing double condenser illumination at 50 kv was used.

RESULTS

Preliminary survey of the effect of LiI on adenovirus type 7. The adenovirus capsid has been disrupted into its individual capsomers by treatment with 6 M LiCl (9), but no further details about the process of capsid degradation have been published thus far. We expected LiI to be more efficient than LiCl. Therefore, the effect of different dosage levels of LiI on the biological activities of virions and soluble viral components was investigated to establish a background for more detailed studies. An approximately 10^5-fold reduction of infectivity titer was achieved when adenovirus was treated with 5.3% (0.4 M) LiI (30 min at 36 C), and a 10^7-fold or greater reduction was achieved with 11% LiI.

When purified adenovirus was treated for 30 min at 36 C with 11% LiI and submitted to rate zonal centrifugation at 24,000 × *g* for 30 min, no CF antigen was recovered in the gradient fractions in which intact virions centrifuged under the same conditions are usually recovered and detected by CF titrations. Instead, increased levels of CF antigen were found in the top fractions of the sucrose gradient, indicating that the capsid of the virus was ruptured. This could be confirmed by electron microscopy of the treated virus (Stasny, *unpublished data*).

Inactivation studies on HA at different concentrations of LiI indicated that IHA was more resistant to LiI than HA (dodecons). Treatment of the latter with 10% LiI for 1 hr at 36 C resulted in complete conversion into IHA. Treatment with

5% LiI was sufficient to inactivate about 90% of HA. LiI failed to affect the hexons at concentrations which caused inactivation of more than 99.9% of HA, 99% of IHA, and a 10^7-fold drop in titer of infectious virus. Significant (i.e., more than twofold) inactivation of hexon antigen was observed at concentrations of LiI exceeding 22%.

Extent of degradation of adenovirus type 7 by LiI at different concentrations of the reagent. When ^{3}H-thymidine-labeled adenovirus type 7 was treated at 36 C with solutions of increasing concentration and submitted to rate zonal centrifugation, the peak of radioactive label recovered in fractions from the gradient, in which intact virus is usually recovered, continuously decreased and more label was recovered in fractions nearer to the top of the gradients. This is exemplified in Fig. 1, which shows representative sedimentation profiles. The lowest concentration of LiI at which the breakdown of virus could be detected was 4%. When the concentration of LiI was doubled, only about 13% of the label was recovered in the region of the gradient corresponding to intact virus. With 22% LiI, the degradation was complete.

It is evident from Fig. 1 that the breakdown of the virus by LiI proceeded through stages, since the labeled degradation products obtained at lower concentrations of the reagent sedimented faster than those obtained by 22% LiI. The virus particles were modified before any changes in their rate of sedimentation became evident; when virus particles were treated with 1.7 and 3.8% LiI, their capacity to adsorb on RBC was reduced to 50 and 25%, respectively, of that observed with intact virus.

Kinetics and temperature dependence of disruption of adenovirus type 7 by LiI. The process of degradation of virus with 11% LiI at 36 C, as determined by the shift in the position of the peak of ^{3}H-thymidine label in sucrose gradients, was completed in about 10 min (Fig. 2) and proceeded through particles sedimenting slower than intact virus but faster than the final product of degradation. This process was strongly dependent on

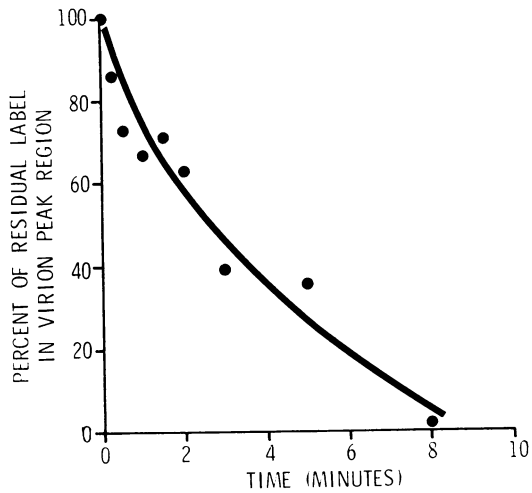

FIG. 2. *Kinetics of disruption of adenovirus type 7 ^{3}H-thymidine-labeled virions were treated with 11% LiI at 36 C for different time intervals and then submitted to rate zonal centrifugation under conditions given for Fig. 1. Fifteen fractions were collected from each gradient. Total radioactivity counts (counts/min) in fractions 7 to 9, in which intact virus occurs, were plotted on the figure.*

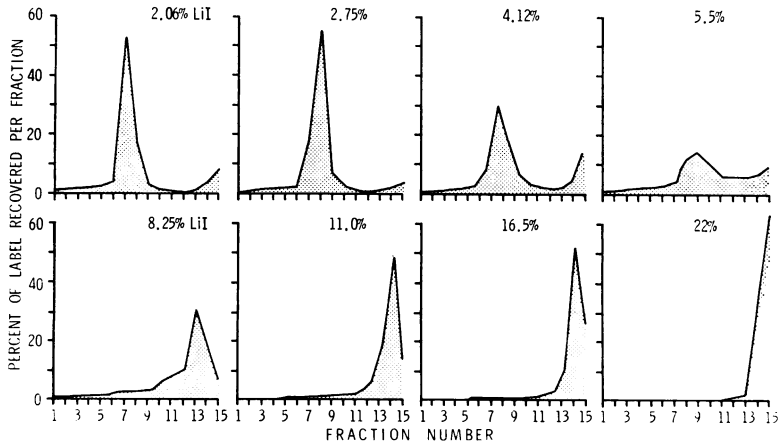

FIG. 1. *Distribution of radioactivity in fractions obtained after rate zonal centrifugation (at 24,000 × g for 30 min) of ^{3}H-thymidine-labeled adenovirus type 7 treated for 30 min at 36 C with solutions containing various amounts of LiI. The sedimentation profile of untreated virus was identical to that obtained with virus treated by 2.06% LiI.*

temperature. No degradation of virus treated with 11% LiI for 5 min was observed between 0 and 15 C. Incubation at higher temperatures initiated the disruption of the virus, which reached 50% at 30 C and was nearly complete at 45 C.

Identification of the final isotopically labeled degradation product obtained by disruption of ^{3}H-thymidine-labeled adenovirus as DNA. Treatment of adenovirus type 7 with formamide leads to the release of nucleoprotein cores from the virus. These cores sediment about three times slower than intact virus but do not stay at the top of the sucrose gradients when centrifuged under conditions given here for Fig. 1 (A. R. Neurath et al., *in preparation*). Since the labeled products that result from degrading adenovirus with labeled DNA by LiI do stay on the top of sucrose gradients under appropriate conditions (Fig. 1), it may be concluded that even viral cores are degraded by LiI. The following results show that DNA free or almost free from protein was released from the virus particles in the ultimate stage of their reaction with LiI.

When virus particles treated with 22% LiI at 36 C for 30 min were submitted to rate zonal centrifugation, the highest portion of the radioactivity was recovered in a position of the gradient corresponding to a sedimentation coefficient ($S_{w,20}$) of about 31, which is equal to the value described for native adenovirus DNA (Fig. 3; 5).

When the same material was submitted to isopycnic density gradient centrifugation in CsCl, the peak of radioactive label was recovered in the fraction, corresponding to a density range of 1.694 to 1.712 g/cm^3 (Fig. 4). To ascertain whether residual protein remained associated with the DNA, virus particles labeled with ^{14}C-amino acids were purified by isopycnic centrifugation, treated with LiI, and submitted to equilibrium density gradient centrifugation under conditions given for Fig. 4. Of the total label, 4.5% was recovered at the density range corresponding to the peak of ^{3}H-thymidine label (Fig. 4). It seems likely that most of this residual ^{14}C label corresponded to nucleic acid bases formed from the labeled amino acids as the result of their metabolic conversion. When DNA was isolated from the same virus preparation by the papain-sodium dodecyl sulfate-phenol method (16), 4.7% of the original ^{14}C label was still associated with DNA.

When the radioactive material recovered from the fractions corresponding to the peak of ^{3}H-thymidine label on Fig. 4 was dialyzed against 0.033 M Tris-acetate-0.033 M EDTA-0.033 M sodium arsenite buffer and then submitted to rate zonal centrifugation under conditions given for Fig. 3, the label again appeared at the same position in the gradient as before. This ruled out the possibility that the peak of radioactivity shown on Fig. 3 might correspond to fragments of DNA attached to protein (which would have a density lower than 1.7 g/cm^3) also having, by coincidence, an $S_{w,20}$ value of about 31.

The ^{3}H-thymidine-labeled material released from the virus was converted from high to low molecular weight material by pancreatic deoxyribonuclease I. On the other hand, exonuclease I, which attacks only single-stranded DNA (10, 23), failed to do so. However, DNA released from the virus and denatured by incubation at 100 C for 5 min was susceptible to exonuclease I (Table 1).

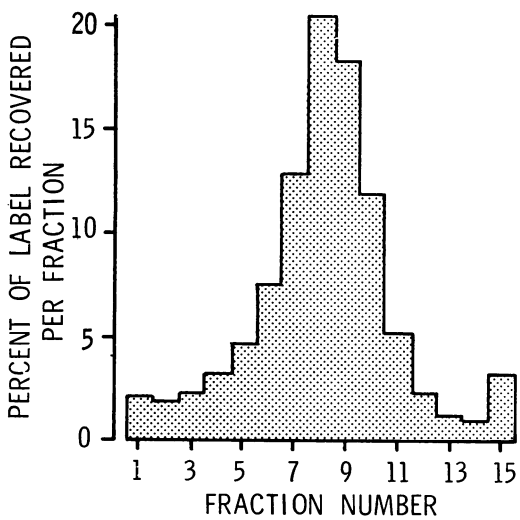

FIG. 3. *Distribution of radioactivity in fractions obtained by rate zonal centrifugation (64,000 × g for 4 hr) of ^{3}H-thymidine-labeled adenovirus type 7 treated with 22% LiI for 30 min at 36 C.*

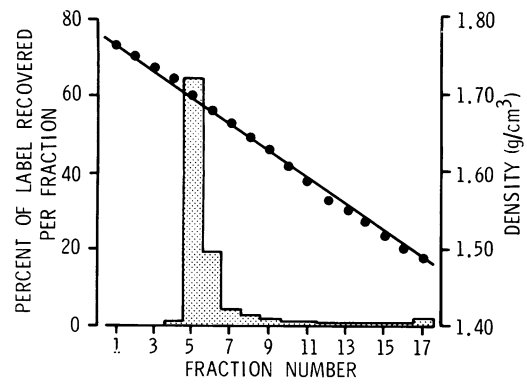

FIG. 4. *Distribution of radioactivity in fractions obtained by isopycnic density gradient centrifugation (at 109,000 × g for 67 hr) of ^{3}H-thymidine-labeled adenovirus type 7 treated with LiI under conditions given for Fig. 3.*

TABLE 1. *Effect of exonuclease I on untreated and heat-denatured adenovirus type 7 DNA released from the virus particles by 22% LiI*

DNA	Units of exonuclease I added	Acid-soluble radioactivity (% of total)
Untreated.....	0	0
Untreated.....	10	1.6
Untreated.....	80	1.2
Heat-denatured....	0	0
Heat-denatured....	10	31
Heat-denatured....	80	86

The DNA released from the virus by LiI, therefore, must have been double-stranded. The results of the described experiments, however, cannot exclude the possibility that DNA strand separation was initiated by LiI and that reannealing occurred during subsequent dialysis.

When fractions from CsCl gradients corresponding to the peak of radioactive label were submitted to electron microscopy, DNA strands were observed. The length of the strands was in the range of 3 to 12 μm. It seems likely that the shorter molecules resulted from breakage during grid preparation. The same was suggested for adenovirus type 7 DNA extracted from the virus by phenol (5).

DISCUSSION

Exposure of adenovirus to solutions of LiI under proper conditions results in the disruption of the viral capsid. This process seems to be initiated at the vertices of the viral icosahedron, since the virus loses its ability to react with cell receptor sites before any disruption of the capsid can be detected by rate zonal centrifugation. Hexons do not seem to be affected by treatment with LiI under conditions sufficient to release DNA from the virus. Considering the recently described antigenic complexity of adenovirus hexon antigen (E. Norrby, Virology, *in press*; E. Norrby, J. Gen. Virol., *in press*), it remains to be established whether LiI would in any way affect the type-specific antigen which is probably responsible for inducing virus-neutralizing antibodies. Preliminary results have shown that LiI-disrupted adenovirus preparations induce virus-neutralizing antibodies.

LiI affects adenovirus in a way distinct from formamide, and the initiation of virus degradation by the two reagents is probably also quite different. Conformational changes in polypeptides induced by lithium halides have been ascribed to specific binding of the salt to peptide linkages (7). It is possible that such binding leads to the disruption of the adenovirus nucleoprotein core into its protein and nucleic acid constituents, a phenomenon not observed with other reagents known to disrupt the viral capsid (8, 12, 14, 17, 19, 20).

DNA released from the virus particles by LiI seems to be in a double-stranded form. This agrees with previous studies (6) showing that the melting point (92.6 C) of sea urchin DNA is decreased in the presence of 4 M LiI by about 14 C. The melting point of adenovirus type 7 DNA is 90.3 C (16), so that its exposure to 22% (1.65 M) LiI at 36 C, i.e., at conditions routinely used for the release of DNA from the virus, probably could not initiate strand separation. It has been suggested that 5 M sodium perchlorate is a suitable reagent for the release of nucleic acids from viruses (3, 4), but this reagent causes a much larger decrease in DNA melting points than LiI at the same molar concentration (6). Further studies are required to characterize in detail the properties of DNA released from adenoviruses by LiI.

The possibility of sequentially cleaving adenovirus into capsomers and the nucleoprotein core (8, 20; A. R. Neurath et al., *in preparation*) and the latter into DNA and protein with LiI offers an advantageous approach for the study of the internal core proteins. This is especially true since these proteins are not completely extracted by acids (17), and their extraction with buffers containing sodium dodecyl sulfate (8, 12, 18) may lead to the irreversible binding of sodium dodecyl sulfate to protein (13) and, therefore, to difficulties in studies of the possible role of these proteins in the transcription of viral DNA.

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